The Orange Carotenoid Protein: Mechanism of a Photoswitch

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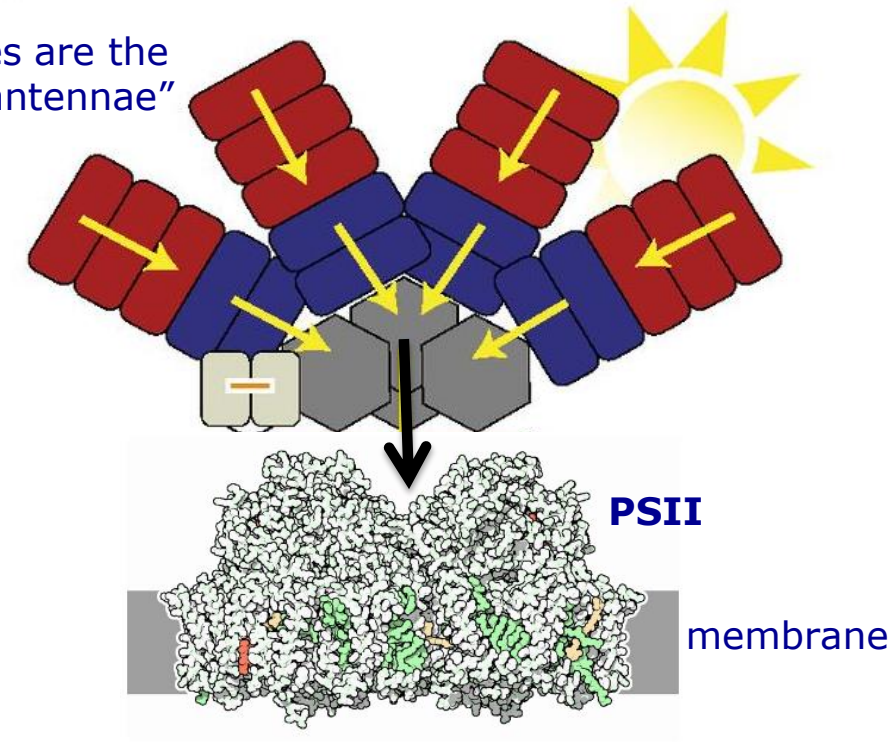
AGENDA

- Context: Why we care about the Orange Carotenoid Protein (OCP)
- What we've learned so far: hybrid methods lead to hypothesis for a photoswitch mechanism
- Future plans for elucidating the bigger system

PHOTOPROTECTION

Photosynthetic organisms turn light into chemical energy through a complex interplay of proteins

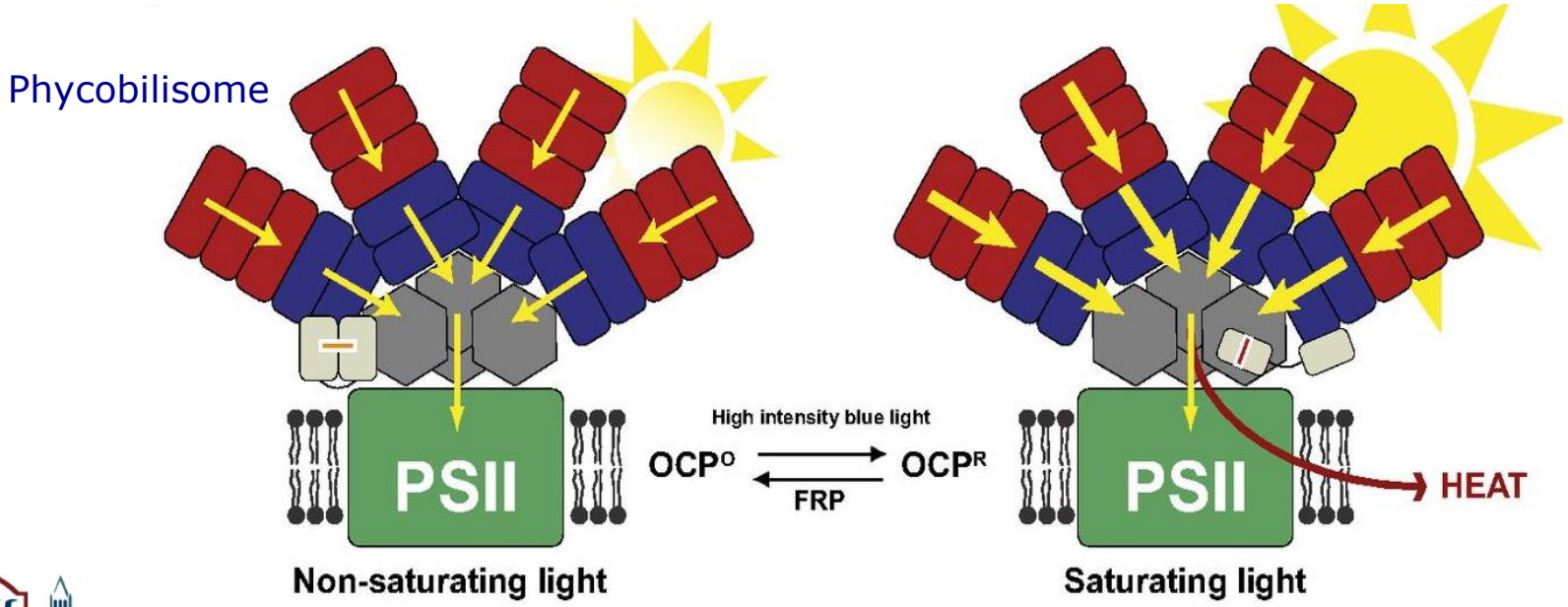
The Phycobilisomes are the light-harvesting "antennae"



PHOTOPROTECTION

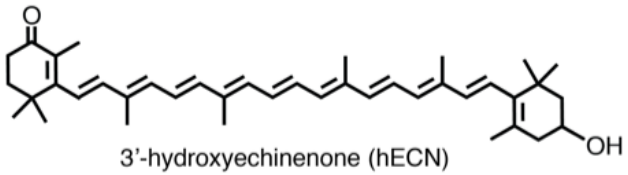
But too much light can damage the organism

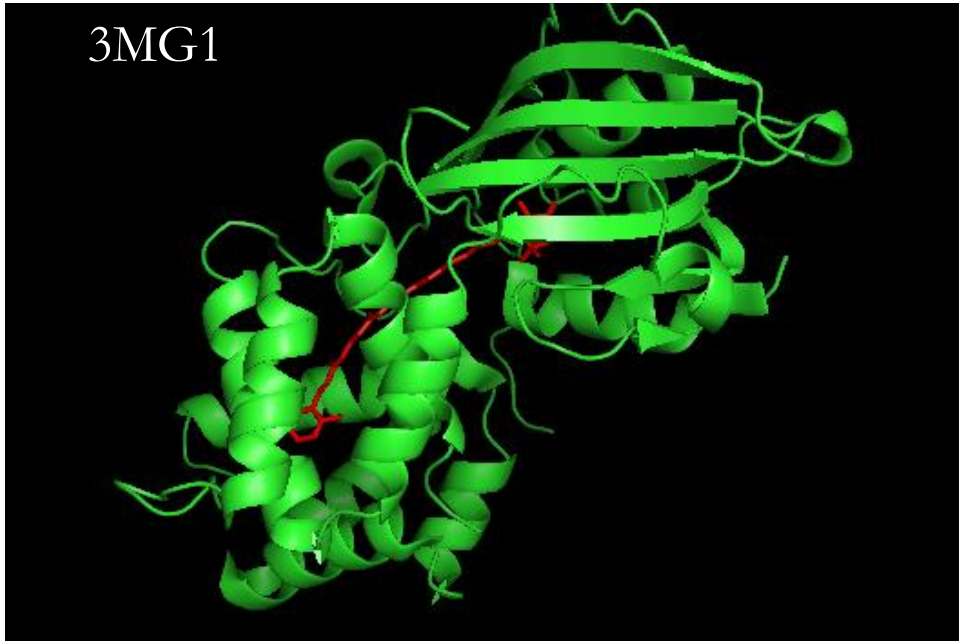
Cyanobacteria have evolved a unique way of dissipating excess energy using the Orange Carotenoid Protein (OCP)



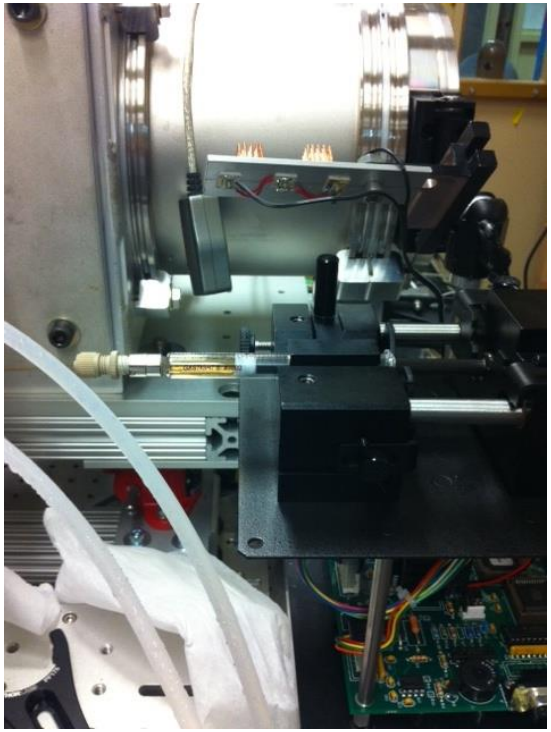
A Key Player: The Orange Carotenoid Protein

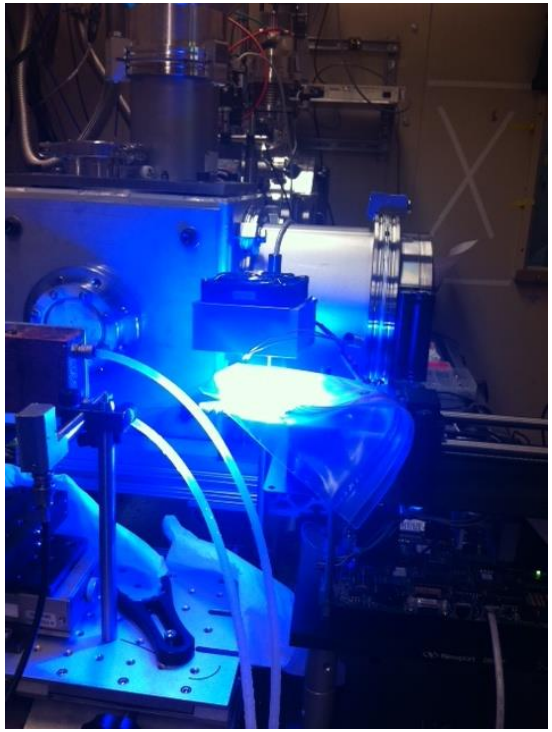
- Activated in cyanobacteria in high light conditions
- Used to quench excess energy
- Contains a light-sensitive pigment carotenoid
- Inactive "orange" form has been crystallized
- Active "red" form has not been crystallized

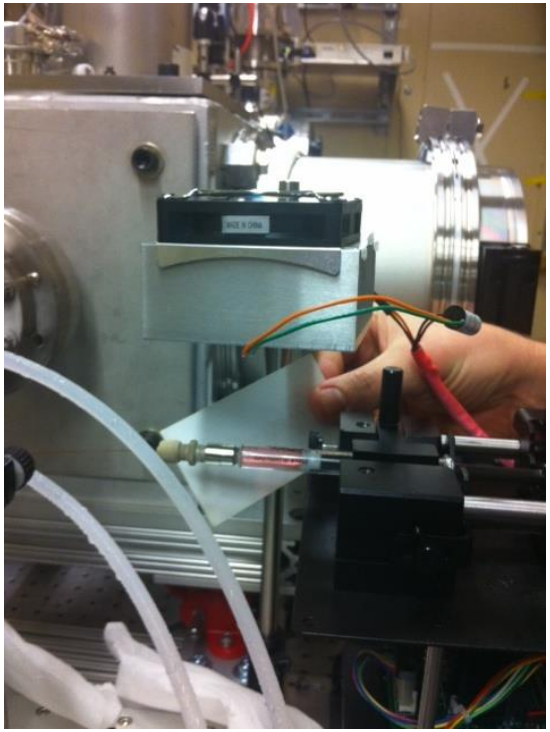




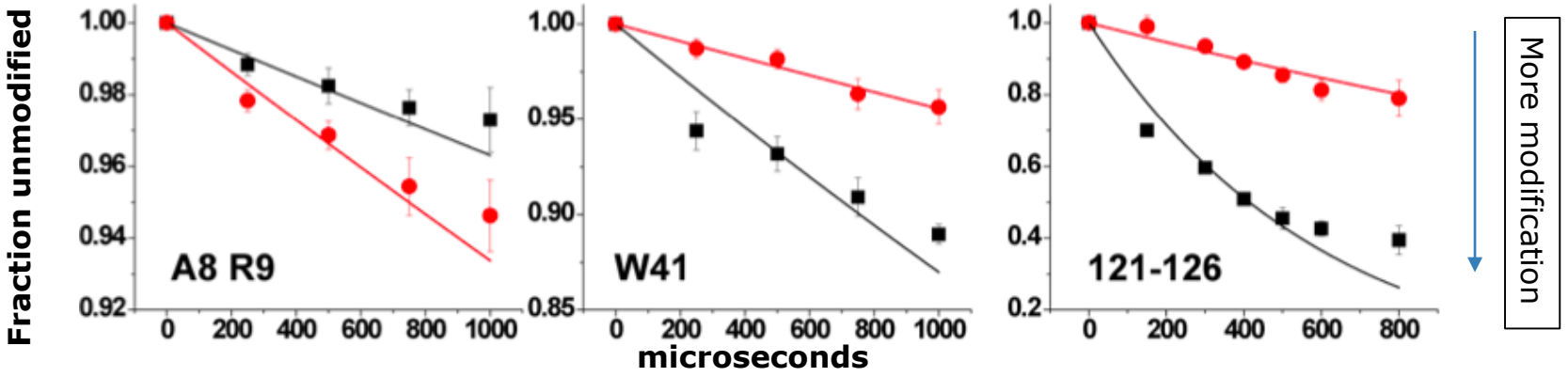
Orange To Red Conversion via Light

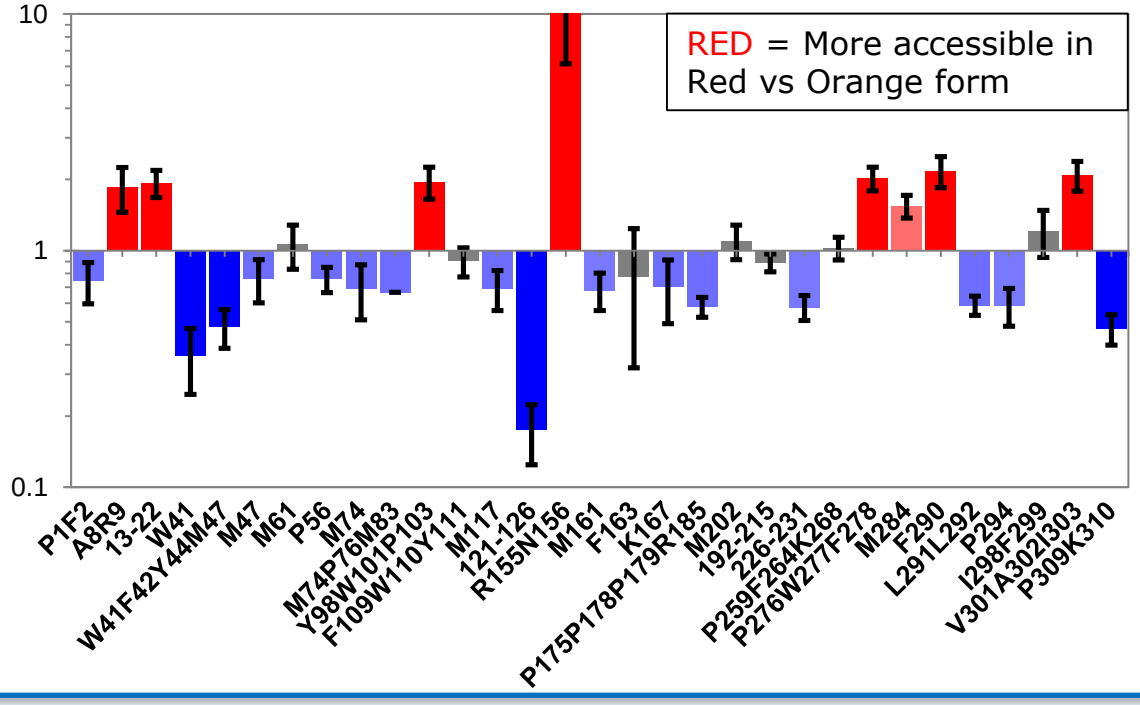




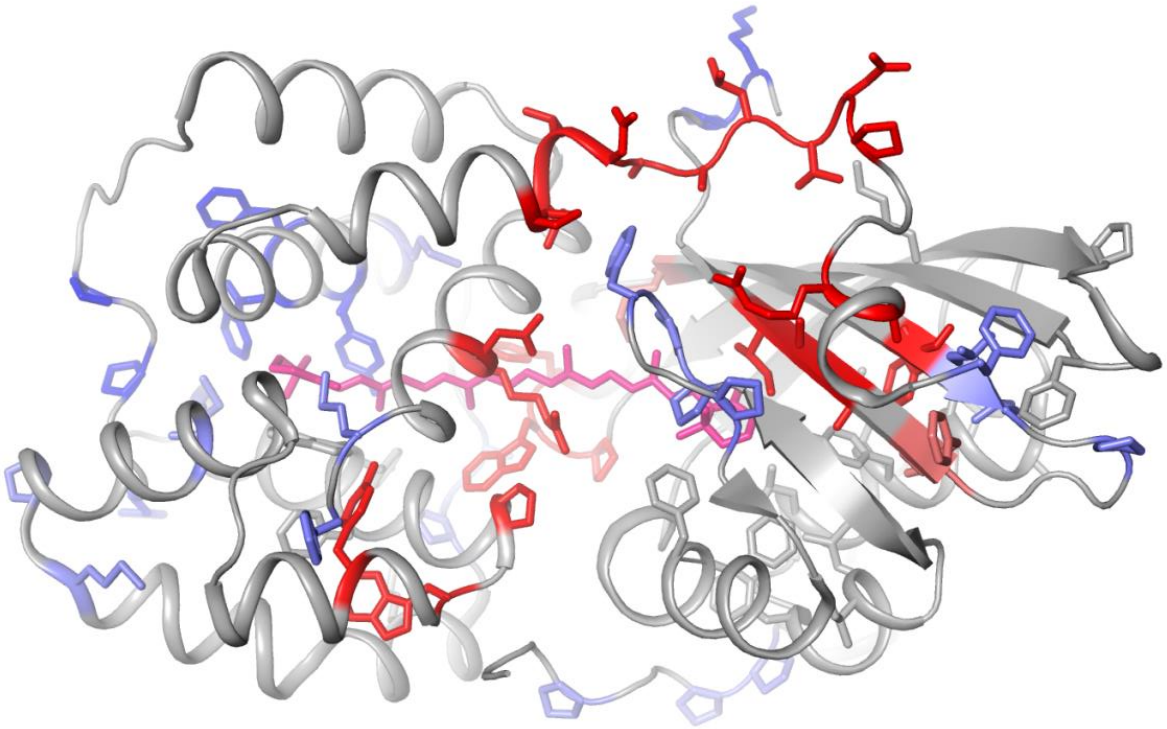


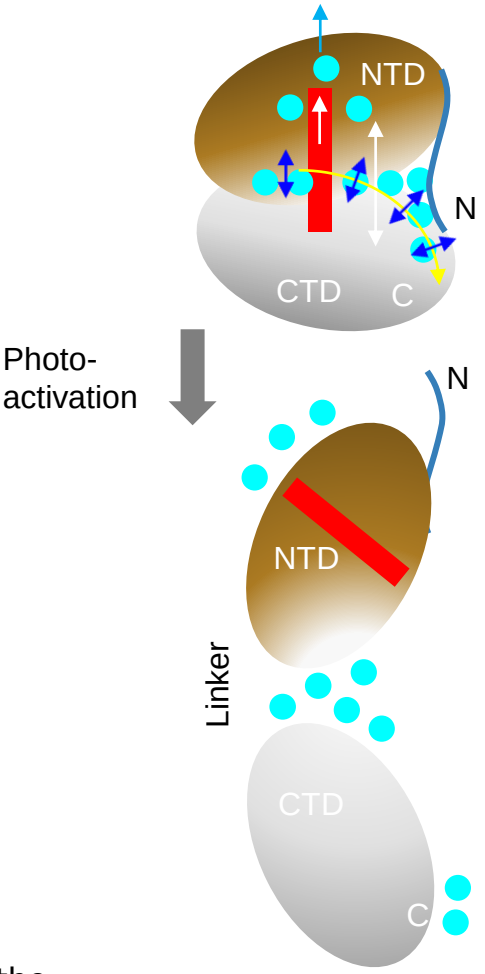
PROTECTIONS OBSERVED USING FOOTPRINTING





Footprinting Data Supports Domain Separation

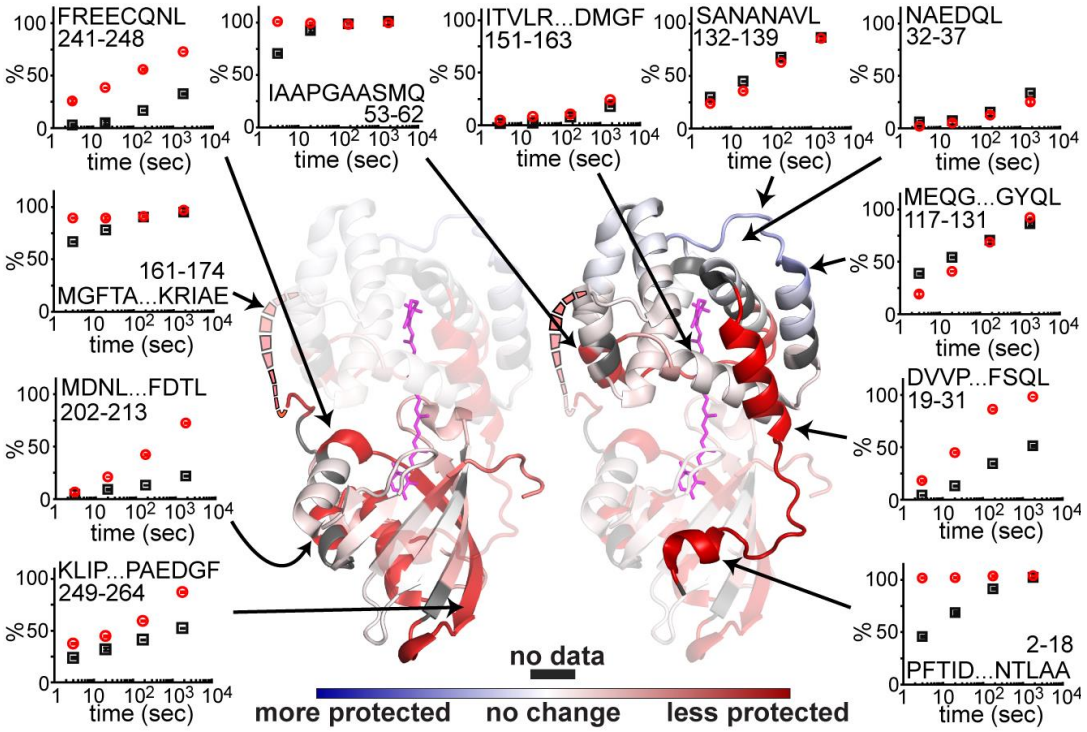




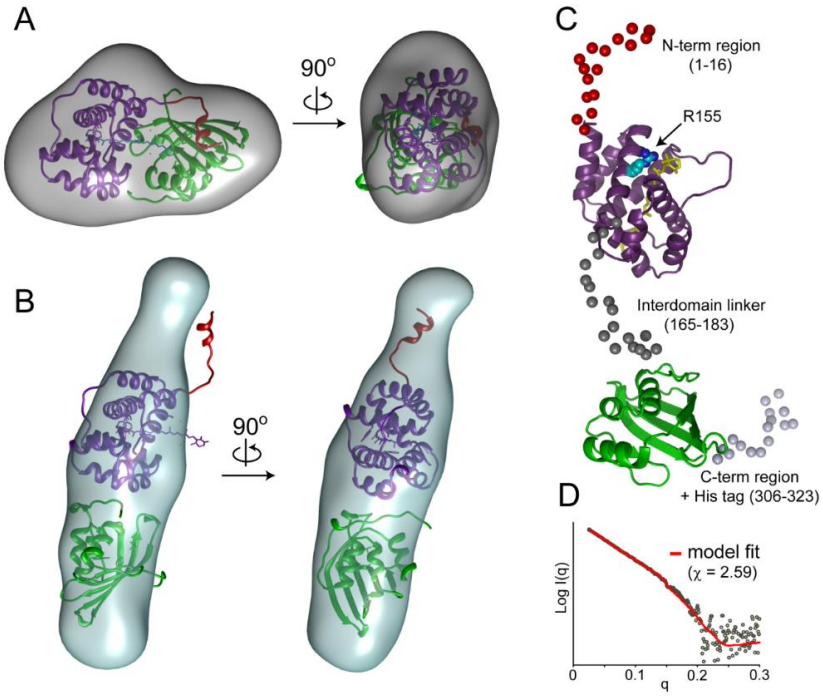
Gupta, Guttman et al, "Local and global structural drivers for the photoactivation of the orange carotenoid protein," *PNAS*, V112 No41, E5567, 2015.

Small Angle Scattering (SAXS) and Hydrogen-Deuterium Exchange (HDX) Data Support Domain Separation

HDX

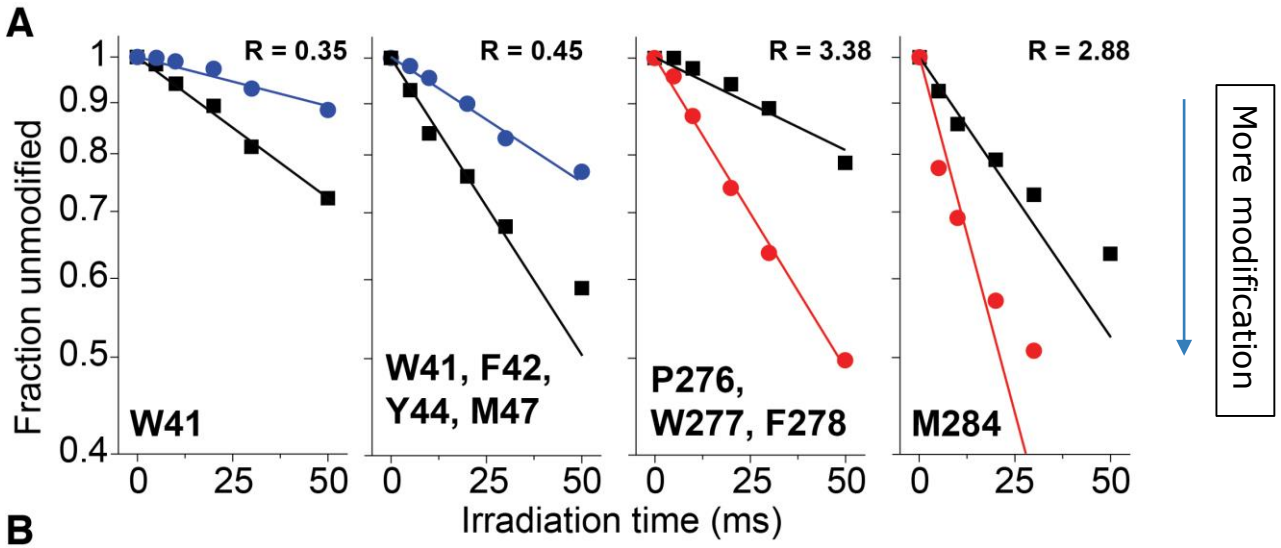


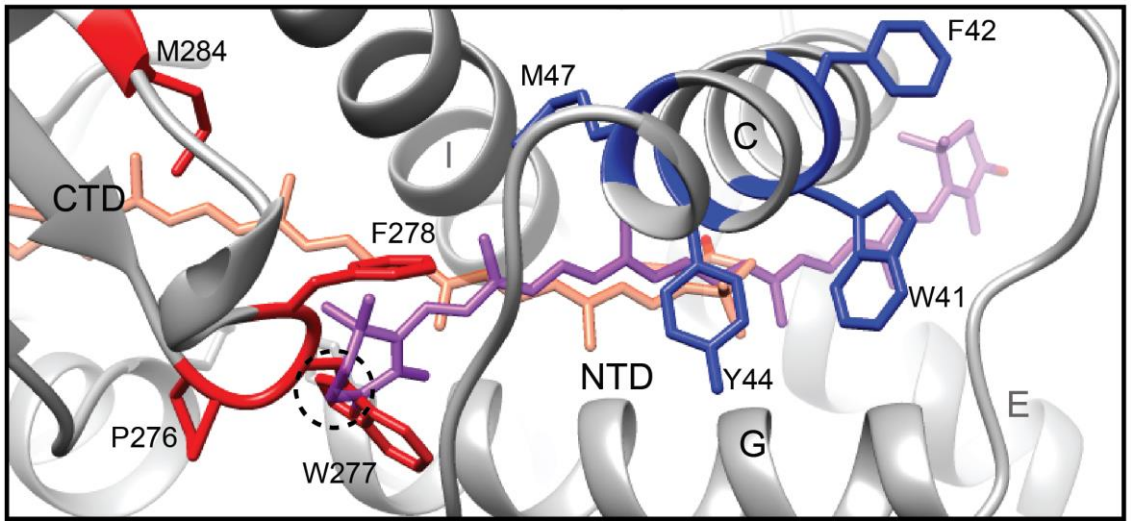
SAXS



The model shows that in OCPR, the NTD and CTD are separated by ~ 16 Å

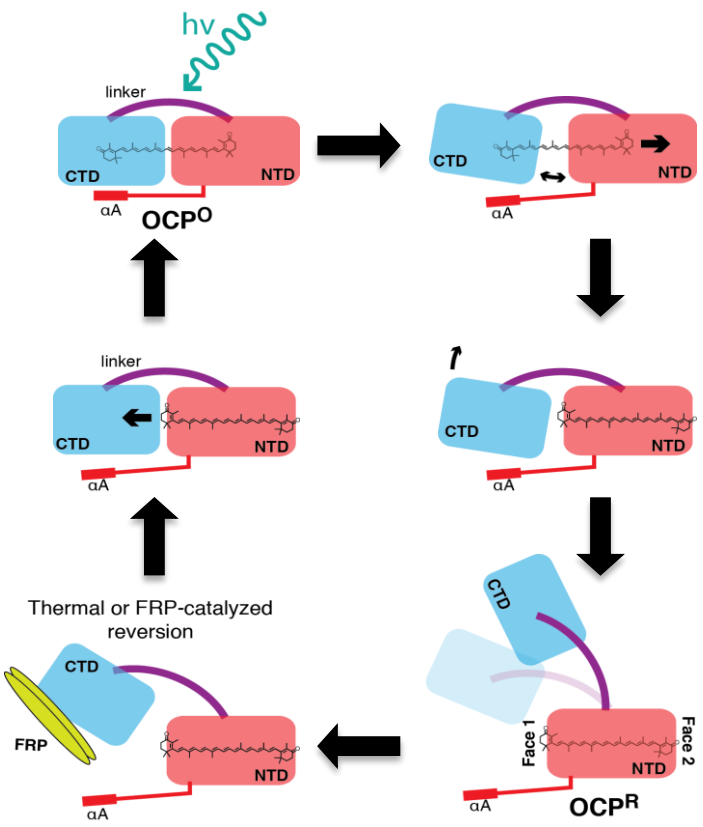
Carotenoid Movement within the Protein

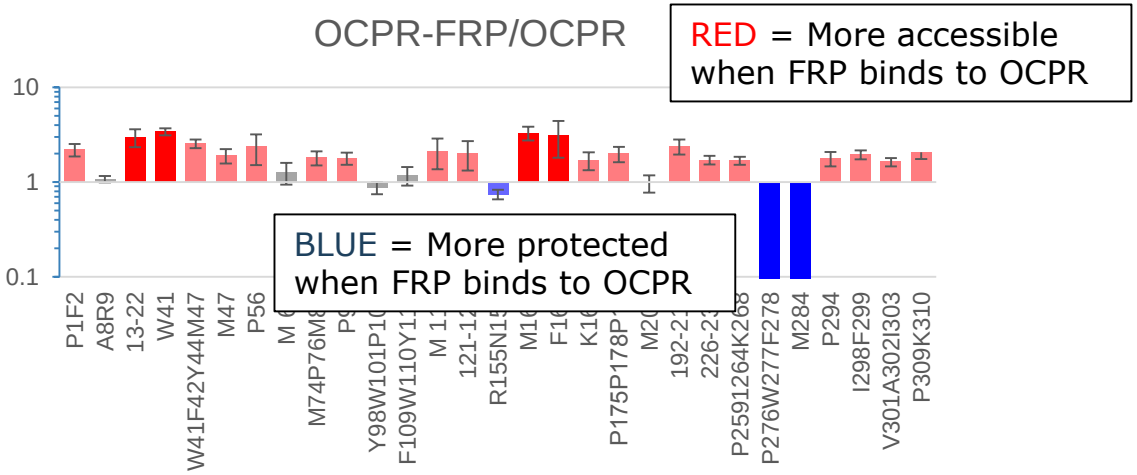


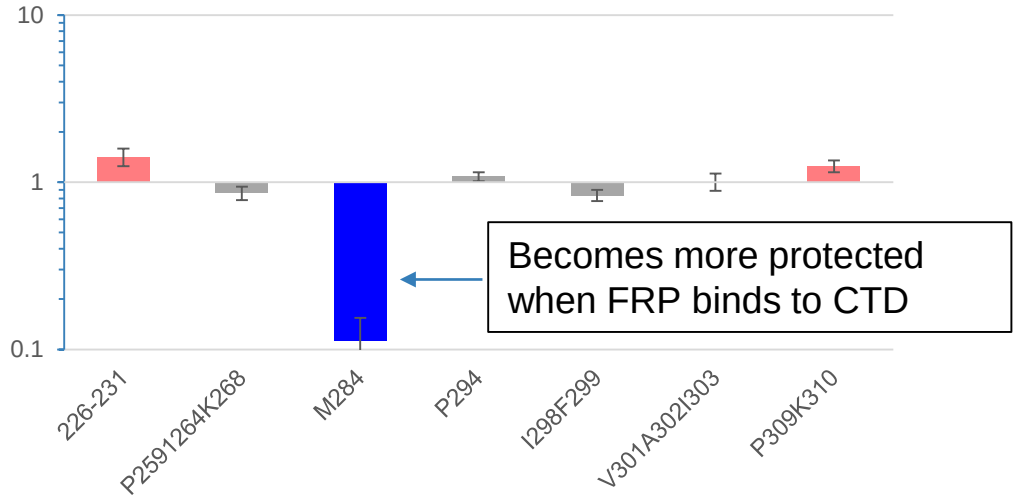


Leverenz, Gupta et al, "Carotenoid translocation in the Orange Carotenoid Protein activates a photoprotective mechanism in cyanobacteria," *Science* V348, 6242, p1463, 2015.

PROTECTION OF OCP WHEN FRP BINDS



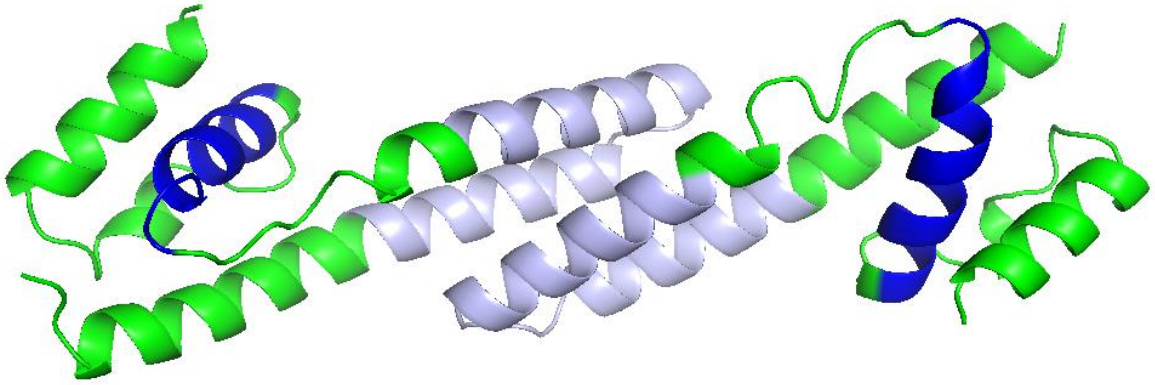




Footprinting From the FRP "point of view"

Peptide	Mods	FRP / OCPO-FRP	FRP / OCPR-FRP (color on structure)
ELDGLLATVQAQA SQITQIDDLWK	Various residues	1	1.5 (light blue)
LHDFLSAK	Various residues	1.4	1.5 (light blue)
QSVIIFVFAQLLK	Predominant at II and LL	1.4	2.1 (dark blue)
EGLVQAEELTFLA ADK	Predominant at EE and F	1	1.2 (didn't plot)

Higher number means more protection upon complex formation

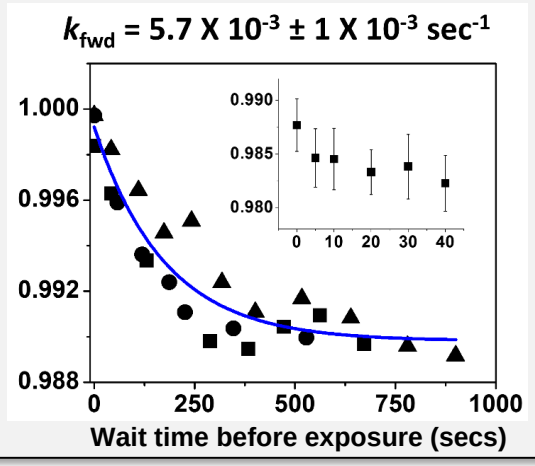


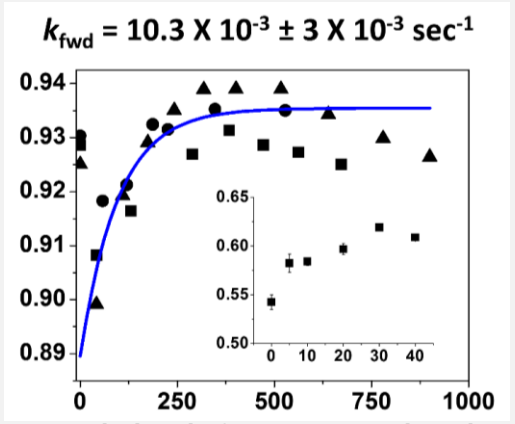
Color	Fold Protec.
	1.5
	2.1

Time Resolved Orange/Red Conversion

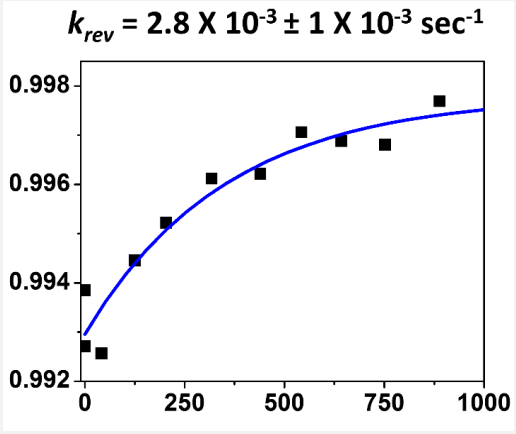
Exposure time constant at: 0.3ms

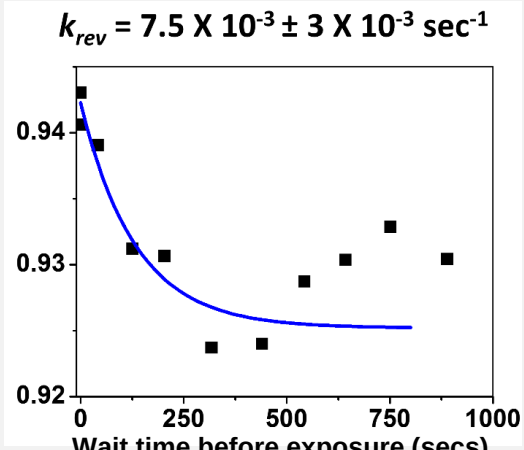
	R155,156	W41, Y44, M47
ORANGE TO RED	k_{fwd} = 5.7 X 10^{-3} ± 1 X 10^{-3} sec^{-1}	k_{fwd} = 10.3 X 10^{-3} ± 3 X 10^{-3} sec^{-1}
RED TO ORANGE	k_{rev} = 2.8 X 10^{-3} ± 1 X 10^{-3} sec^{-1}	k_{rev} = 7.5 X 10^{-3} ± 3 X 10^{-3} sec^{-1}





More modification



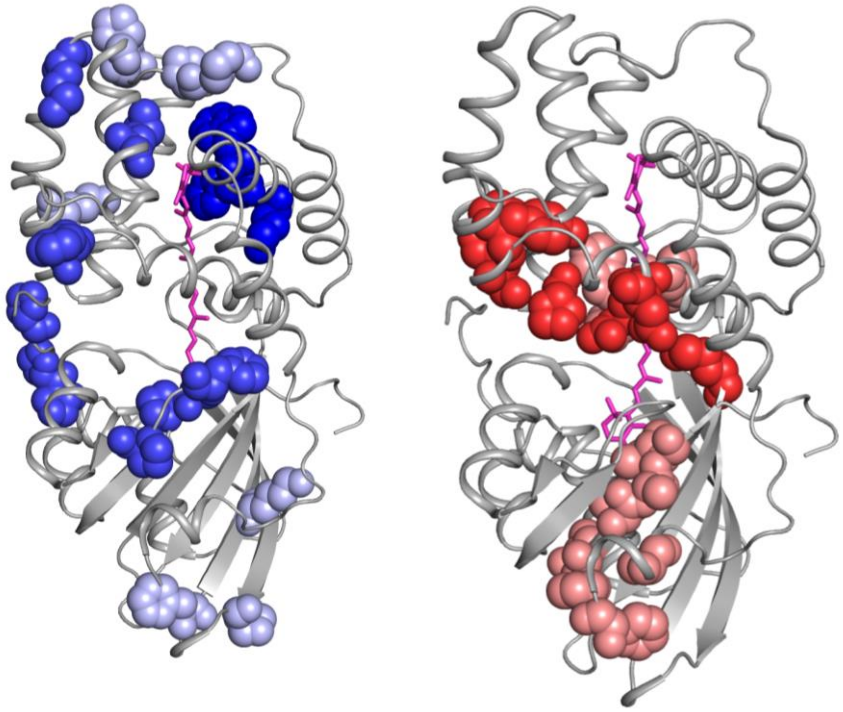


More modification

Pictoral View of Orange-Red Conversion

Decrease in SA

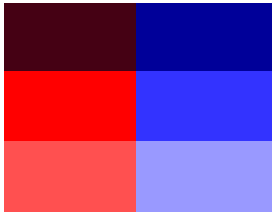
Increase in SA



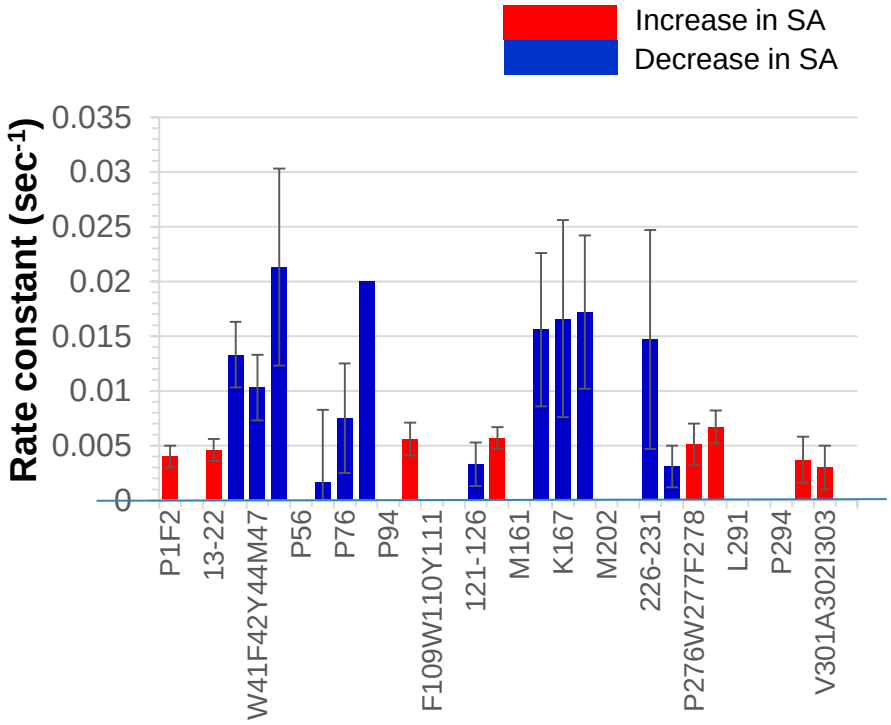
> 0.010 s^{-1} (Fast)

< 0.010 to > 0.005 s^{-1}

> 0.005 s^{-1} (Slow)



OCP^O to OCP^R Conversion



What's Next?

- Repeat the kinetics experiments
- Investigate FRP light sensitivity
- Further investigate FRP-OCP interactions
- Investigate OCP-PB interactions